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Low-Dose Estrogen Supplementation Improves Vascular Function in Hypogonadal Men

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Abstract—It is widely accepted that in women, estrogens provide protection against the development of cardiovascular disease. However, the cardiovascular role of estrogens in men remains uncertain, despite preliminary evidence that endogenous estrogens produced by aromatization of androgenic precursors are of physiological importance. Hypogonadal men have very low levels of circulating estrogen. We studied the responsiveness of forearm resistance arteries to vasoconstrictor and vasodilator agents in 12 men (mean±SEM age, 68.7±2.6 years) rendered hypogonadal as a result of treatment for prostatic cancer, before and after 8 weeks of estrogen supplementation (estradiol valerate 1 mg daily; n=7) or placebo (n=5). Forearm blood flow was measured by venous occlusion plethysmography, and vasoactive agents were infused through a brachial artery cannula in doses that did not affect blood pressure or heart rate. Estrogen supplementation was well tolerated, with no adverse effects. After estrogen treatment, mean estradiol levels increased from <30 to 308±65 pmol/L, and both systolic and diastolic blood pressures were reduced. HDL cholesterol levels increased significantly, and vasoconstrictor responses to the NO synthase inhibitor N^G-monomethyl-L-arginine (1, 2, 4 μmol/min) were enhanced. Vasoconstrictor responses to angiotensin II (8, 16, 32 ng/min) were markedly attenuated by estrogen treatment, as were vasoconstrictor responses to norepinephrine (25, 50, 100 ng/min). Estrogen did not alter the vasodilator responses to acetylcholine (9.25, 18.5, 37 μg/min) or to the endothelium-independent agent sodium nitroprusside (1.6 μg/min). Responses to all vasoactive agents were unchanged after administration of placebo. We conclude that low-dose estrogen supplementation in hypogonadal men is well tolerated, lowers blood pressure, and may affect vascular reactivity in a manner that is potentially beneficial, through several mechanisms, including enhancement of basal NO release and attenuation of vasoconstrictor responses to angiotensin II and norepinephrine. These findings suggest the need to consider a possible clinical role for estrogenic compounds in cardiovascular risk reduction in some groups of men. **(*Hypertension*. 2001;38:1011-1016.)**

Key Words: estrogen ■ men ■ vascular diseases ■ hypogonadism ■ nitric oxide ■ norepinephrine ■ angiotensin II ■ stress

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Women are relatively protected against cardiovascular disease in comparison with men. Many observational studies have suggested that estrogen treatment of postmenopausal women significantly reduces cardiovascular risk[1]; however, the recently concluded Heart Estrogen/Progestin Replacement Study (HERS) found no significant evidence of a clinical benefit of hormonal therapy in women with established coronary artery disease.[2] In men, estrogens are produced in significant quantities by local tissue aromatization of androgenic precursors from the testes and adrenal glands.[3] There has been relatively limited study of the biological role of these hormones or their clinical implications in men.

In human endothelial cells, which reportedly have a high density of estrogen receptors (20 to 80 000 per cell), the intensity of immunostaining for estrogen receptors is similar in male and female donor cells, and neither electrophoretic mobility shift assays nor ligand-binding studies show reproducible gender differences in estrogen receptor expression.[4] Endothelial dysfunction[5] and coronary artery disease[6] have been reported in a young male with an absence of functional estrogen receptors, suggesting a role for estrogen in cardiovascular health in men. However, an investigation conducted 25 years ago into the cardiovascular effects of estrogen administration in men after myocardial infarction—the Coronary Drug Project—showed an excess of deaths and recurrent infarction in the treatment group. This trial, which used high doses of conjugated equine estrogens, was subsequently abandoned, and the subject has not been studied in detail since.[7]

In recent years, it has been increasingly recognized that high doses of estrogens may produce effects contrary to those obtained at physiological levels. Furthermore, greatly en-

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hanced knowledge is now available regarding the wide range of actions of estrogens on the cardiovascular system, even in male tissues.[8] These developments suggest that it may be appropriate to reassess the adverse findings of the Coronary Drug Project. To pursue this, we have investigated the cardiovascular effects of low-dose estrogen supplementation in a group of men rendered hypogonadal as a result of treatment for prostatic cancer, thereby reducing endogenous estrogen production. In a small, randomized, placebo-controlled study, we have examined the effects of 8 weeks of estrogen supplementation on a number of surrogate cardiovascular end points, including lipid levels, blood pressure, and forearm vascular reactivity.

Methods

Subjects

We studied 12 men rendered hypogonadal by surgical or pharmacological treatment for prostatic cancer. We excluded patients with clinical evidence of cardiovascular disease (or on cardiovascular drugs) or severe renal, hepatic, respiratory, or hematologic conditions. The study was approved by the Alfred Hospital Human Ethics Committee. All subjects gave written fully informed consent.

Study Design

The study followed a randomized, double-blind, placebo-controlled design. Subjects were randomly assigned to 8 weeks of estrogen supplementation with estradiol valerate (Progynova, Schering) 1 mg daily (n=7; mean age, 68.4±2.6 years) or placebo (n=5; mean age, 69.2±2.8 years). Hemodynamic and forearm vascular studies were performed twice, 8 weeks apart. Subjects were unaware of treatments received. All measurements were made by investigators blinded to the treatment. On each study day, subjects underwent the following procedures: arterial cannulation, blood sampling, assessment of forearm vascular reactivity, and measurement of hormones and other variables.

Arterial Cannulation

Subjects rested supine throughout each study in a quiet, temperature-controlled room, (22°C). The left brachial artery was cannulated with a 21-gauge, 5-cm catheter (Cook) for intra-arterial blood pressure measurement (Spacelabs Inc), drug infusions, and arterial blood sampling. Heart rate was monitored by electrocardiography. Subjects rested for 30 minutes before commencement of the study.

Blood Sampling

Blood was taken on each occasion for measurement of urea, electrolytes, glucose, total cholesterol, triglycerides, HDL cholesterol, liver function, full blood count, estradiol, testosterone, androstenedione, dehydroepiandrosterone, sex hormone–binding globulin, follicle-stimulating hormone (FSH), and luteinizing hormone (LH).

Assessment of Forearm Vascular Reactivity

Forearm vascular responsiveness was assessed by venous occlusion plethysmography with a sealed alloy-filled gallium and indium double-stranded strain gauge (Medasonic). Hand blood flow was excluded via a wrist cuff (200 mm Hg); venous occlusion pressure was 50 mm Hg. Basal blood flow was obtained as an average of 3 measurements. Drugs were infused via an infusion pump at 2 mL/min.

Acetylcholine (BDH Chemicals) was infused at 9.25, 18.5, and 37 μg/min; norepinephrine at 25, 50 and 100 ng/min; and angiotensin (Ang) II at 8, 16, and 32 ng/min, each dose for 2 minutes to allow blood flow to reach steady state. Basal NO release was assessed by intra-arterial infusion of N^G-monomethyl-L-arginine (L-NMMA) (Calbiochem-Novabiochem) at 1, 2, and 4 μmol/min, each dose for 5 minutes. Finally, sodium nitroprusside (David Bull Laboratories) was infused at 1.6 μg/min for 2 minutes. The peak response was determined as the average of 3 consecutive steady state measurements. A 15-minute rest period between interventions was sufficient for flow to return to resting levels.

TABLE 1. Baseline Characteristics of Subjects Randomized to Estrogen (n=7) and Placebo (n=5)

Treatment Group	Age, y	Weight, kg	BMI, kg/m^2	Systolic BP, mm Hg	Diastolic BP, mm Hg
Estrogen	68.4±2.1	72.4±4.5	27.2±2.0	136±1	87±1
Placebo	69.2±2.8	70.8±4.9	27.6±2.3	134±1	86±2

BMI indicates body mass index; BP, blood pressure.

Measurement of Hormones and Other Variables

Estradiol and other hormones, namely, total testosterone, androstenedione, FSH, LH, and sex hormone–binding globulin were measured by specific radioimmunoassay.[9] The intra-assay coefficient of variation for estradiol was 9% (n=32) and sensitivity 30 pmol/L. Total cholesterol, triglycerides, HDL cholesterol, and glucose were also measured.

Calculations and Statistical Analysis

Results are expressed as mean±SEM. Vascular reactivity dose-response curves were compared by 2-way repeated-measures ANOVA. Other data were compared by Student's *t* test. In cases in which multiple comparisons were made, the Bonferroni correction was applied. The null hypothesis was rejected at $P<0.05$.

Results

There were no significant adverse effects reported in any of the subjects. Only 1 subject experienced symptoms likely to be associated with estrogen treatment: mild, persistent breast tenderness. The other subjects were unable to tell whether they were receiving active treatment or placebo.

Baseline Characteristics

Baseline characteristics are shown in Tables 1 and 2. There were no differences between the estrogen- and placebo-treated groups with respect to age, body mass index, systolic or diastolic blood pressure, or baseline plasma levels of estradiol, total testosterone, androstenedione, sex hormone–binding globulin, LH, total cholesterol, triglycerides, HDL cholesterol, hemoglobin, or glucose. FSH was higher in the placebo group as a result of very high levels in 1 individual. Testosterone levels were very low in both estrogen- and placebo-treated groups, consistent with their hypogonadal status.

Effect of Estradiol on Serum and Plasma Measurements

As expected, estrogen levels increased with estrogen treatment, FSH levels fell, and androgen levels showed no change. HDL levels increased significantly with estrogen treatment, but total cholesterol and triglyceride levels were constant (Table 2). Glucose levels were unchanged with estrogen treatment. There were no significant differences in the placebo group. No changes were observed in renal function, liver function, or any hematologic parameters.

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TABLE 2. Hormone and Lipid Levels in Each Group Before and After Administration of Either Estrogen or Placebo

Treatment Status	17β-Estradiol, pmol/L (<283)	Total Testosterone, nmol/L (6.9–28.1)	Androstenedione, nmol/L (2–6)	SHBG, U/L (13–71)	FSH, U/L (1–8)	LH, U/L (2–12)	Cholesterol, mmol/L (<5.5)	Triglycerides, mmol/L (<2.0)	HDL Cholesterol, mmol/L (>1.0)	Hemoglobin, g/L (135–170)	Glucose, mmol/L (4–6)
Estrogen group											
Before estrogen	<30	<1	1.6±1.4	46.6±11.2	32.2±10.6	12.7±3.8	5.8±0.4	1.9±0.4	0.96±0.12	132.8±3.4	6.0±0.4
After estrogen	308±65*	<1	0.6±0.3	46.8±10.4	20.5±8.6†	12.9±3.4	5.6±0.5	1.5±0.2	1.04±0.13†	128.6±3.8	5.8±0.3
Placebo group											
Before placebo	<30	<1	0.8±0.3	41.0±4.9	73.6±11.8	29.3±8.8	5.5±0.2	2.2±0.5	1.08±0.07	131.3±6.2	5.8±0.7
After placebo	<30	<1	0.4±0.1	47.5±8.6	63.6±29.2	32.5±10.5	5.1±0.3	2.7±0.7	1.00±0.09	128.0±5.9	6.1±0.4

SHBG indicates sex hormone–binding globulin. Normal values for men are shown within parentheses at the top of each column. Results are expressed as mean±SEM.

*$P<0.001$, †$P<0.05$ vs baseline values. Estrogen treatment was associated with increases in plasma estradiol and HDL cholesterol and decreases in FSH and glucose. Values were unchanged after treatment with placebo.

Effect of Estradiol on Acetylcholine-Induced Vasorelaxation

Acetylcholine induced a dose-dependent increase in forearm blood flow. The acetylcholine dose-response relationship did not change significantly after supplementation with either estrogen or placebo (Figure 1A and 1B). Blood pressure and heart rate were unchanged during acetylcholine infusions, at baseline, and after both estrogen and placebo administration.

Effect of Estradiol on L-NMMA–Induced Vasoconstriction

L-NMMA induced a dose-dependent decrease in forearm blood flow. After estradiol supplementation, the degree of vasoconstriction induced by L-NMMA was considerably enhanced (Figure 1C; $P<0.01$ from 2-way ANOVA, before versus after estradiol), suggesting an increase in basal NO release. The L-NMMA dose-response relationship after administration of placebo did not change significantly (Figure 1D). Blood pressure and heart rate were unchanged during L-NMMA infusions at baseline and after estrogen or placebo administration.

Effect of Estradiol on Norepinephrine-Induced Vasoconstriction

Norepinephrine induced a dose-dependent decrease in forearm blood flow. After estrogen supplementation, the degree of vasoconstriction induced by norepinephrine was attenuated ($P=0.03$ from 2-way ANOVA, before versus after estrogen), suggesting a decrease in noradrenergic responsiveness (Figure 2A). There was no significant change in the norepinephrine dose-response relationship after administration of placebo (Figure 2B). Blood pressure and heart rate were

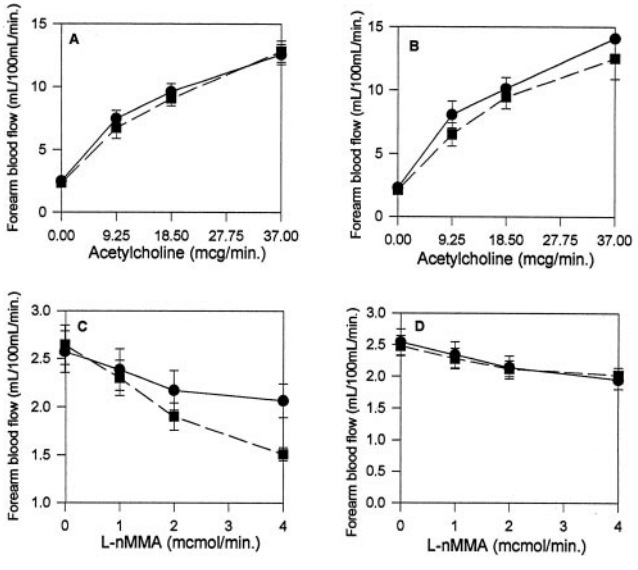

Figure 1. Responses to acetylcholine and L-NMMA. Endothelium-dependent vasodilation induced by acetylcholine did not change after treatment with either estrogen (A) or placebo (B). However, vasoconstriction induced by the nitric oxide synthase inhibitor L-NMMA was markedly accentuated in the subjects receiving estrogen (C), suggesting that in this group basal nitric oxide release was significantly enhanced. No significant change occurred in responses of the placebo group (D). ●, Values before treatment with estrogen or placebo; ■, values after treatment.

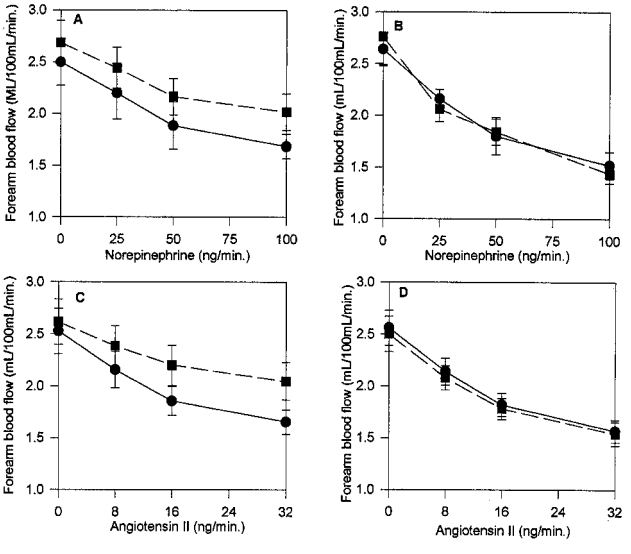

Figure 2. Responses to norepinephrine and Ang II. Vasoconstrictor responses to norepinephrine were significantly attenuated after treatment with estrogen (A), whereas no change occurred with placebo (B). Vasoconstrictor responses to Ang II were significantly accentuated after treatment with estrogen (C), with no significant change in the responses of the placebo group (D). ●, Values before treatment with estrogen or placebo; ■, values after treatment.

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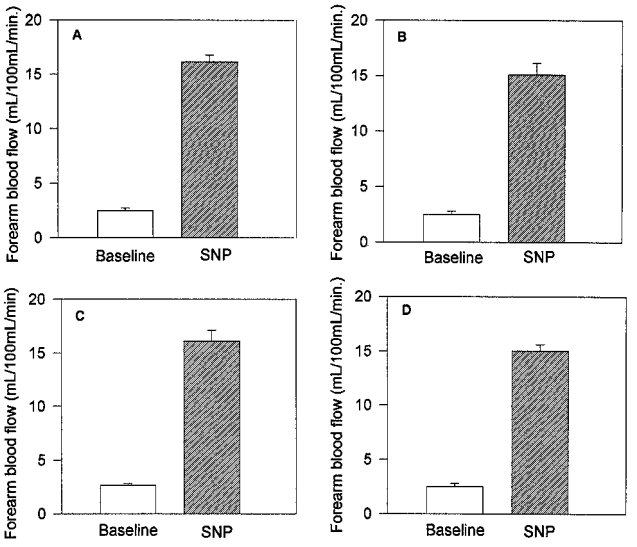

Figure 3. Responses to sodium nitroprusside (SNP). Vasodilator responses to SNP were unaffected by treatment with either estrogen (before estrogen [A] and after estrogen [B]) or placebo (before placebo [C] and after placebo [D]).

unchanged during norepinephrine infusions, at baseline, and after estrogen or placebo administration.

Effect of Estradiol on Ang II–Induced Vasoconstriction

Ang II induced a dose-dependent decrease in forearm blood flow. After estrogen supplementation, the degree of vasoconstriction induced by Ang II was attenuated (Figure 2C; $P=0.04$). There was no significant change in the Ang II dose-response relationship after administration of placebo (Figure 2D). Blood pressure and heart rate were unchanged during Ang II infusions, at baseline, and after estrogen or placebo administration.

Effect of Estradiol on Sodium Nitroprusside–Induced Vasodilation

There was no change in the responses to the endothelium-independent vasodilator sodium nitroprusside after administration of either estrogen (Figure 3A and 3B) or placebo (Figure 3C and 3D). Blood pressure and heart rate were unchanged during nitroprusside infusions at baseline and after estradiol or placebo administration.

Effect of Estradiol Supplementation on Resting Blood Pressure

In subjects receiving estrogen supplementation, there was a significant drop in both systolic (before estrogen, 136 ± 1 mm Hg; after estrogen, 132 ± 1 mm Hg; $P<0.025$) and diastolic (before estrogen, 87 ± 0.3 mm Hg; after estrogen, 84 ± 0.3 mm Hg; $P<0.05$) blood pressures. In subjects who received placebo, there was no significant change in either systolic (before placebo, 134 ± 1 mm Hg; after placebo, 133 ± 1 mm Hg; $P=$NS) or diastolic (before placebo, 85 ± 1 mm Hg; after placebo, 84 ± 1 mm Hg; $P=$NS) blood pressures.

Discussion

This randomized, double-blind, placebo-controlled study shows that low-dose estrogen supplementation in hypogonadal men is well tolerated and influences a range of cardiovascular parameters; namely, it increases plasma HDL cholesterol levels, enhances basal NO release, attenuates vasoconstrictor responses to norepinephrine and Ang II in forearm resistance arteries, and reduces systolic and diastolic blood pressure.

Our study suggests a beneficial effect of estrogens on the cardiovascular system in hypogonadal men. It is well established that HDL cholesterol exerts antiatherosclerotic effects, and low HDL cholesterol levels are associated with higher rates of coronary artery disease.[10] NO is believed to retard atherogenic processes such as smooth muscle proliferation,[11] monocyte adhesion,[12] and platelet aggregation,[13] and it is recognized that endothelium-dependent relaxation is impaired in patients with hypercholesterolemia, hypertension, and coronary artery disease.[14] Norepinephrine appears to act as a vasoconstrictor under conditions of stress and in some cases of hypertension, and increased noradrenergic tone has been implicated in the pathogenesis of hypertension and congestive heart failure.[15] Activation of the renin-angiotensin system has been implicated in vascular[16] and cardiac disease.[17] Thus, estradiol-induced changes in the cardiovascular parameters measured in this study represent potentially beneficial effects on the cardiovascular system.

The role of estrogens in reducing cardiovascular risk in women has been the subject of extensive investigation over many years, with many studies showing favorable effects of estrogens on lipids and other surrogate markers of cardiovascular disease. Nonetheless, it is important to recognize that controversy continues about the role of hormonal therapy in both primary and secondary prevention of cardiovascular disease in women.[1,2,18] The single placebo-controlled prospective study of hormonal therapy for secondary prevention of coronary artery disease in women—the aforementioned HERS investigation[2]—not only failed to demonstrate a reduction in coronary events over a 4-year period but showed an increase in thromboembolic events in the treatment group in comparison with placebo.

Similarly, in men, there are conflicting data about the cardiovascular effects and possible clinical roles of estrogens.[8] In the only prospective, randomized, placebo-controlled trial with clinical end points, the Coronary Drug Project, neither the higher (5 mg) nor the lower (2.5 mg) dose of conjugated equine estrogens reduced the risk of recurrent coronary heart disease events.[7] However, it is now generally accepted that this study used estrogen doses many-fold higher than those used in hormonal therapy today and at least 8-fold higher than that used in the present study. By contrast, as in women, studies using more "physiological" doses of estrogen and involving surrogate end points have generally suggested potentially beneficial effects of estrogens in men. Acute intravenous administration of conjugated estrogens appeared to improve coronary blood flow responses to acetylcholine[19] and abolished abnormal coronary vasoconstriction in response to an exogenous cold stimulus both in men referred for routine coronary angiography[20] and in male cardiac

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allografts.[21] Furthermore, studies in male-to-female transsexuals have shown that both flow-mediated and nitroglycerin-induced vasodilations in the brachial artery are enhanced compared with control men, suggesting that high-dose estrogen treatment enhances vascular reactivity in genetic males.[22,23] In healthy young men, single doses of sublingual estradiol or intravenous conjugated equine estrogens, at plasma concentrations within the physiological range for premenopausal women, have been shown to induce rapid onset, rapid offset, nongenomic effects on the endothelium-mediated vasodilator response to acetylcholine, with no effect on the endothelium-independent action of sodium nitroprusside.[24] Not all studies have yielded positive results: for example, a study of short-term estrogen supplementation failed to show an effect on acetylcholine-induced coronary artery vasoconstriction in men,[25] and estrogens reportedly did not augment flow-mediated dilation in the brachial artery or influence serum levels of metabolites of NO in older male subjects.[26] Of interest, in vitro studies of 17β-estradiol–induced vasodilation in human epicardial coronary arteries have shown an attenuated response in male compared with female patients, and neither NO synthase nor cyclooxygenase inhibition influenced this response.[27]

The present study considered hypogonadal men, who have low levels of endogenous estrogen production and who with low-dose estrogen supplementation show increases in plasma estradiol levels from very low to modest levels, comparable to those in found in premenopausal women. The results are broadly consistent with those found in perimenopausal and postmenopausal women. The attenuation of norepinephrine-induced vasoconstriction after estrogen supplementation is similar to our observation in perimenopausal women[28] and, as previously suggested,[29] may be related to an estrogen-induced decrease in α-adrenoceptor binding. As in this study, estrogen has previously been reported to decrease Ang II–induced vasoconstriction in experimental animals[30] and in human internal mammary arteries in vitro, most likely through a direct effect on vascular smooth muscle cells.[31] A decreased response to Ang II is likely to represent a potentially beneficial effect of estrogen with respect to cardiovascular risk. Of interest, a decrease in Ang II–induced vasoconstriction was not observed in our previous study in perimenopausal women.[28] In the present study it is also possible that estrogen-induced increase in basal nitric oxide release (previously demonstrated in perimenopausal women[32]) resulted in a decreased basal arterial tone and thus an attenuated vasoconstrictor responsiveness to both norepinephrine and Ang II. Androgen withdrawal has been shown to enhance endothelium-dependent vasodilation in adult men[33]; in our study, however, testosterone levels were equally low in both the estrogen- and placebo-treated groups.

In the present study, as in our previous studies in women,[28,32] estrogen supplementation induced a fall in both systolic and diastolic blood pressure, but no change occurred in the group that did not receive estrogen. Previous studies in women examining the effect of estrogens on blood pressure have been conflicting, with some studies showing an increase in blood pressure,[34,35] others showing no change,[18,36,37] and yet others suggesting an antihypertensive effect.[38–40] Differences in the type of estrogen administered[39] or in dose[41] might help to explain some of the discrepancies observed in these earlier studies in women. Nevertheless, it would appear from our study that in hypogonadal men 17β-estradiol exerts blood pressure–lowering effects.

In summary, our results show that, without producing significant adverse effects, short-term treatment with low-dose estrogen in elderly hypogonadal men leads to changes in cardiovascular reactivity of a potentially beneficial nature. Accordingly, we conclude that, at least in this group of patients, there may be a role for low-dose estrogen supplementation in the management of cardiovascular risk. We recognize that in our study estrogen induced an improvement in a number of surrogate end points, namely, enhanced endothelium-dependent vasorelaxation, attenuated responses to vasoconstrictors, and reduced blood pressure. We therefore suggest that to explore further the possible physiological roles of estrogen and to examine the potential therapeutic use of estrogenic compounds in men at high risk of cardiovascular disease, further studies that emphasize clinical end points are warranted.

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